was measured by a liquid scintillation soln consisting of PPO (0.4 g), POPOP (0.06 g) in toluene (100 ml) or of naphthalene (10 g), PPO (0.7 g) and POPOP (0.05 g) in dioxane (100 ml).

Feeding experiment. 1) An aq. soln of DL-phenylalanine-[3-^{14}C] (50 μCi, 1.46 × 10^{10} dpm/mM) was administered to the plant (60–70 cm, high) by cotton wick method and after the feeding for 48 hr leaves were cut off, washed, chipped and exhaustively extracted with MeOH. MeOH extract (3.96 × 10^7 dpm) suspended in Et_2O was separated into Et_2O soluble (Fr. 1) and $NaHCO_3$ soluble (Fr. 2) fractions. The Et_2O fraction (Fr. 1) was saponified with 5% KOH–EtOH for 0.5 hr refluxing and after the removal of an unsaponifiable fraction aq. layer was acidified with 10% HCl and extracted with Et_2O. The Et_2O extract was purified by preparative TLC to afford phyllodulcin, hydrangenol and umbelliferone. An alkaline fraction (Fr. 2) was acidified with 10% HCl and extracted with Et_2O to give *p*-hydroxybenzoic acid, protocatechuic acid and gallic acid. The H_2O layer was extracted with BuOH and after the removal of non-glycosidic substances, such as gallic acid, by preparative TLC, the glycosidic fraction was hydrolyzed with 5% H_2SO_4 for 1 hr and the hydrolysate was extracted with Et_2O to give aglycones. Aglycones were purified by preparative TLC to afford phyllodulcin, hydrangenol, umbelliferone, kaempferol and quercetin. Parallel feeding experiment using cinnamic acid-[3-^{14}C] (25 μCi, 1.01 × 10^{10} dpm/mM) was carried out. The MeOH extract (2.03 × 10^7 dpm) was separated to Fr. 1 and Fr. 2, and was carried out in the same way. The metabolites obtained from each feeding experiment were identified by dilution method and the results are summarized in Table 1. Also an aq. soln of *p*-coumaric acid-[U-^{3}H] (9.90 × 10^6 dpm, 2.64 × 10^9 dpm/mM), caffeic acid-[U-^{3}H] (1.13 × 10^7 dpm, 3.73 × 10^9 dpm/mM) or hydrangea glucoside A-[U-^{3}H] (2.95 × 10^6 dpm, 5.87 × 10^8 dpm/mM) was separately fed to cut plants. After feeding for 29 hr, each plant was extracted with MeOH and the MeOH extract [*p*-coumaric acid-[U-^{3}H] (5.98 × 10^6 dpm); caffeic acid-[U-^{3}H] (4.81 × 10^6 dpm); hydrangea glucoside A-[U-^{3}H] (2.85 × 10^6 dpm)] was chromatographed on polyamide by elution with MeOH–H_2O (4:1). The phenolic metabolites were separated by preparative TLC into acid-flavonoid, glycoside and dihydroisocoumarin fractions. The acid-flavonoid fraction was separated by preparative TLC to give *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, quercetin and rutin. Gylcosidic fraction was hydrolyzed with β-glucosidase and the aglycones extracted with Et_2O were separated by preparative TLC to afford hydrangenol and phyllodulcin. The fraction consisting of dihydroisocoumarins was separated by preparative TLC to give umbelliferone, hydrangenol and phyllodulcin. The metabolites obtained from each feeding experiment were identified by dilution method (see Table 2).

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ANTITUMOR AGENT FROM *JUNIPERUS BERMUDIANA* (PINACEAE): DEOXYPODOPHYLLOTOXIN

BAHMAN TAMMAMI, STERLING J. TORRANCE and JACK R. COLE*
College of Pharmacy, University of Arizona, Tucson, AZ 85721, U.S.A.

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Key Word Index—*Juniperus bermudiana*; Pinaceae; lignan; deoxypodophyllotoxin.

As a result of the continuing search for plants having tumor-inhibitory constituents, it was found that the EtOH extract of the twigs and leaves of *Juniperus bermudiana* L. (Pinaceae)† showed inhibitory activity toward the P-388 lymphocytic leukemia (PS) and cytotoxic activity toward the human epidermoid carcinoma of the nasopharynx (KB) test systems of the Division of Cancer Treatment, National Cancer Institute, N.I.H., Bethesda, MD. *Juniperus bermudiana* L. has not been previously examined but other *Juniperus* species have been shown to contain lignans [1], including deoxypodophyllotoxin [2]. We now wish to report that deoxypodophyllotoxin has been isolated from *J. bermudiana* and indeed was the agent solely responsible for the cytotoxic activity.

The ethanol extract of the defatted, dried and ground twigs and leaves of *Juniperus bermudiana* was partitioned between $CHCl_3$ and H_2O (1:1). The $CHCl_3$ phase, after evaporation of the solvent, was treated with hexane. The hexane-soluble portion was extracted with 5% $NaHCO_3$ and the neutral portion was chromatographed on a Si gel 60 (E. Merck) column. The separation was monitored at all stages by testing the fractions obtained in the KB test system. Thus the

* To whom inquiries should be directed.

† The plant was collected in Hawaii in February, 1972. Identification was confirmed by Dr. Robert E. Perdue, Chief, Medicinal Plant Resources Laboratory, U.S.D.A., Beltsville, MD. A reference specimen is maintained by the U.S.D.A.

active column fraction was further purified by successive preparative TLC's until the pure KB-active material had been isolated. This compound was identified as the lignan deoxypodophyllotoxin by spectral (IR, NMR) analysis, mp and comparison with an authentic specimen (undepressed mmp, identical IR spectra).

Deoxypodophyllotoxin has been found in our laboratories [3] to exhibit PS activities of 194% test/control (T/C) and 161% T/C at dose levels of 12.5 and 6.25 mg/kg, respectively. Activity in the PS test system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C $\geqslant$ 125% [4]. In the KB test system, deoxypodphyllotoxin exhibited an activity of 0.00024 μg/ml. Activity in the KB test system is defined as $ED_{50} \leqslant 20$ μg/mg [5].

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OSAJAXANTHONE FROM *KIELMEYERA CORIACEA*

J. L. C. LOPES*, J. N. C. LOPES†, B. GILBERT‡ and SUZELEI E. BONINI†

* Faculdade de Filosofia Ciências e Letras de Ribeirão Preto; † Núcleo de Pesquisas de Produtos Naturais da Faculdade de Farmácia e Odontologia de Ribeirão Preto, Ribeirão Preto, S.P., Brasil; ‡ Centro de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, ZC 32, Brasil

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Key Word Index—*Kielmeyera coriacea*; Guttiferae; osajaxanthone; schistosomicide.

The extract of *K. coriacea* Mart. in hexane–EtOAc (4:1) showed protection against infection by cercariae of *Schistosoma mansoni*, when applied to the skin of experimental animals. This extract was fractionated by column chromatography over Si gel and afforded a yellow compound, whose physical data were compatible with osajaxanthone (2,2-dimethyl-5,8-dihydroxypyrano-[3,2-b]xanthone). Osajaxanthone exhibited the protective activity against *S. mansoni* cercariae observed in the extract. This compound has been isolated previously from *Maclura pomifera* Raf. (Moraceae) [1], *Calophyllum scriblitifolium* Hend & Wyatt Smith [2], *C. canum* Hook (Guttiferae) [3], *K. corymbosa* (Spr.) Mart [4] and *K. ferruginea* A. P. Duarte [5]. Acetylation of osajaxanthone gave the colourless diacetate whose NMR spectrum [1] and mp confirmed the identity of the parent compound. This is the first report of a xanthone possessing significant schistosomicidal activity.

EXPERIMENTAL

NMR and MS were made by Dr. Paul M. Baker (Federal University of Rio de Janeiro), mps are uncorr.

Osajaxanthone. Pulverized wood and leaves of *K. coriacea* (7 kg) were extracted at room temp. with hexane–EtOAc (4:1), giving, after removal of the solvent, 43 g of a dark brown gum. 30 g of this gum was chromatographed over Si gel (750) g in hexane–EtOAc giving crude osajaxanthone (150 mg), which separated from EtOAc as yellow needles; mp 264–266°, lit. 264–265° [1], $FeCl_3$, green; UV λ_{max}^{EtOH} 240, 249, 288, 340 and 382 nm (log ε 4.2, 4.2, 4.6, 3.8, 3.6); MS (high resolution) showed M^+ at *m/e* 310.08389 (calc. for $C_{18}H_{14}O_5$, 310.084116). A mmp with an authentic sample showed no depression. *Osajaxanthone diacetate.* Crystallized from EtOH as needles, mp 200°, lit. 203–204° [1]; NMR ($CDCl_3$, 60 MHz) δ 1.40 (*s*, 6H), 2.40 (*s*, 3H), 2.47 (*s*, 3H), 5.75 (*d*, 1H), 6.42 (*d*, 1H), 6.70 (*s*, 1H), 7.40 (*m*, 2H) and 7.90 (*m*, 1H), MS (70 eV) *m/e* 394 (M^+, 8%).

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